

WHAT IS CLAIMED IS:

1. A method for identifying an immunosuppressive agent comprising:

(a) obtaining at least one population of viable cultured active T cells having intact cell membranes from a cell growth medium under conditions conducive to growth;

(b) combining a first portion of said at least one population with a predetermined amount of at least one test compound dissolved in a solvent for a predetermined period of time at a predetermined temperature thereby generating a first volume;

(c) combining a second portion of said at least one population with an amount of the solvent which was used to dissolve said at least one test compound, for said predetermined period of time at said predetermined temperature thereby generating a second volume;

(d) separately adding to each of said first volume and said second volume a reporter compound having at least one measurable property which is responsive to the caspase cascade;

(e) measuring said at least one measurable property of said reporter compound in said first volume and thereby measuring the caspase cascade activity of said first volume;

(f) measuring said at least one measurable property of said reporter compound in said second volume and thereby measuring the caspase cascade activity of said second volume;

(g) calculating a first ratio of caspase cascade activity measured for said first volume to said caspase cascade activity measured for said second volume, wherein when the first ratio is greater than one, said at least one test compound kills active T cells and is identified as a potential immunosuppressive agent.

T02030"3223560

2. The method of claim 1, further comprising:

(a) obtaining at least one population of viable cultured resting T cells having intact cell membranes from a cell growth medium under conditions conducive to growth;

(b) combining said resting T cells with said predetermined amount of said identified immunosuppressive agent dissolved in said solvent for said predetermined period of time at said predetermined temperature thereby generating a third volume;

(c) adding to said third volume said reporter compound having at least one measurable property which is responsive to the caspase cascade;

(d) measuring said at least one measurable property of said reporter compound in said third volume and thereby measuring the caspase cascade activity of said third volume; and,

(e) calculating a second ratio of caspase cascade activity measured for said first volume to said caspase cascade activity measured for said third volume, wherein when the second ratio is greater than one, then said identified immunosuppressive agent is further identified as an active-T-cell-selective immunosuppressive agent.

3. The method of claim 1 or 2, further comprising measuring said at least one measurable property using fluorescence methodology, colorimetric assays, absorbance spectroscopy or direct visualization techniques.

4. The method of claim 1 or 2, wherein said at least one test compound is applied to the T cells at a concentration in the range from about 1 picomolar to about 1 millimolar.

5. The method of claim 1 or 2, further comprising adding a permeabilization enhancer in combination with said reporter compound.

6. The method of claims 1 or 2, wherein said predetermined period of time is about 1 minute to about 48 hours; and wherein said predetermined temperature is about 4 °C to about 42 °C.

7. The method of claim 6, wherein said predetermined period of time is about 24 hours to about 48 hours.

8. The method of claim 3, wherein said reporter compound is selected from the group consisting of:

(a) a fluorogenic compound that produces fluorescence under the influence of the caspase cascade;

(b) a chromogenic compound that produces light absorption under the influence of the caspase cascade; and

(c) chemiluminescent compound that produces light emission under the influence of the caspase cascade.

9. The method of claim 1, wherein a plurality of viable cultured active T cell samples are exposed separately to a plurality of test compounds.

10. The method of claim 2, wherein a plurality of viable cultured resting T cell samples are exposed separately to a plurality of test compounds.

11. The method of claims 9 or 10, wherein said plurality of viable cultured cells are in separate wells of a microtiter plate.

12. The method of claim 1, wherein said active T cells are obtained by adding to T cells antibodies to the T cell receptor, Concanavalin A, or Phytohaemagglutinin.

13. The method of claim 1 or 2, wherein said active T cells are obtained from tissue of a patient afflicted with one or more immunopathological symptoms and wherein said resting T cells are from healthy tissue that is not afflicted with the immunopathological symptoms.



identified immunosuppressive agent is further identified as an active-T-cell-selective immunosuppressive agent.

16. The method of claim 14 or 15, further comprising assessing cell viability by observing mitochondrial activity, membrane intactness, or cell number.

17. The method of claim 16, further comprising measuring said mitochondrial activity, membrane intactness, or cell number by using fluorescence methodology, colorimetric assays, or direct visualization techniques.

18. The method of claim 14 or 15, wherein said at least one test compound is applied to the T cells at a concentration in the range from about 1 picomolar to about 1 millimolar.

19. The method of claim 14 or 15, further comprising adding a permeabilization enhancer in combination with said reporter compound.

20. The method of claim 14 or 15, wherein said predetermined period of time is about 1 minute to about 48 hours; and wherein said predetermined temperature is about 4 °C to about 42 °C.

21. The method of claim 20, wherein said predetermined period of time is about 24 hours to about 48 hours.

22. The method of claim 17, further comprising observing said mitochondrial activity, membrane intactness, or cell number using a reporter compound selected from the group consisting of:

(a) a fluorogenic compound that produces fluorescence under the influence changes in mitochondrial activity, membrane intactness, or cell number;

(b) a chromogenic compound that produces light absorption under the influence of changes in mitochondrial activity, membrane intactness, or cell number; and

(c) a chemiluminescent compound that produces light emission under the influence of changes in mitochondrial activity, membrane intactness, or cell number.

23. The method of claim 14, wherein a plurality of viable cultured active T cell samples are exposed separately to a plurality of test compounds.

24. The method of claim 15, wherein a plurality of viable cultured resting T cell samples are exposed separately to a plurality of test compounds.

25. The method of claim 23 or 24, wherein said plurality of viable cultured cells are in separate wells of a microtiter plate.

26. The method of claim 14, wherein said active T cells are obtained by adding to T cells antibodies to the T cell receptor, Concanavalin A, or Phytohaemagglutinin.

27. The method of claim 14 or 15, wherein said active T cells are obtained from tissue of a patient afflicted with one or more immunopathological symptoms and wherein said resting T cells are from healthy tissue that is not afflicted with the immunopathological symptoms.

28. A method for assaying the potency of a test compound to synergise with a known immunosuppressant by functioning as an activator of the caspase cascade, said method comprising:

(a) obtaining at least one population of viable cultured active T cells having intact cell by culturing T cells in a cell growth medium under conditions conducive to growth and activating the cells;

(b) exposing a first portion of said at least one population to a combination of a predetermined amount of said test compound and a subinducing amount of said known immunosuppressant for a first



31. A method for identifying an immunosuppressive agent comprising:

- (a) obtaining viable cultured active T cells having an intact cell membrane;
- (b) obtaining viable cultured resting T cells having an intact cell membrane;
- (c) separately exposing the active and resting T cells to at least one test compound for a predetermined period of time under predetermined conditions;
- (d) adding a reporter compound having at least one measurable property which is responsive to the caspase cascade to the active and resting T cells that have been exposed to the at least one test compound;
- (e) measuring the caspase cascade activity in the active T cells exposed to the at least one test compound by measuring said at least one measurable property; and
- (f) measuring the caspase cascade activity in said resting T cells exposed to the at least one test compound by measuring said at least one measurable property, wherein when the caspase cascade activity in the active cells is greater than the caspase cascade activity in the resting cells, the at least one test compound selectively kills active T cells and is an immunosuppressive agent.

32. A method of treating an immunopathological disease comprising administering to an animal in need of such treatment an effective amount of an immunosuppressive agent, or a pharmaceutically acceptable salt or prodrug thereof, with the proviso that said immunosuppressive agent was first identified according to the method of claim 1.

33. The method of claim 32, wherein said animal is a human.

34. A kit for performing the method of claim 32, comprising packaging material containing said immunosuppressive agent, or a pharmaceutically acceptable salt or prodrug of said immunosuppressive agent,



said packaging material further comprising a label that indicates that said immunosuppressive agent, or a pharmaceutically acceptable salt or prodrug of said immunosuppressive agent, is useful to treat an immunopathological disease.

35. A pharmaceutical composition for treating an immunopathological disease comprising a first immunosuppressive agent, or a pharmaceutically acceptable salt or prodrug of said first immunosuppressive agent, a known second immunosuppressive agent, and a pharmaceutically acceptable vehicle, with the proviso that said first immunosuppressive agent was first identified as an immunosuppressive agent according to the method of claim 1, and wherein said first and second immunosuppressive agents are present in amounts effective to treat the immunopathological disease.

09020334 080201